UDK 616-022.8/.9-07
Review
Received: 14 December 2007
Accepted: 20 February 2008

CURRENT LABORATORY DIAGNOSIS OF ALLERGY

Slavica Dodig

Srebrnjak Children's Hospital, Reference Center for Clinical Pediatric Allergology of the Ministry of Health and Social Welfare, Zagreb, Croatia

Summary

The requests for laboratory diagnosis of allergic diseases refer to a battery of tests that should provide the following: 1) determine the type of allergic reaction by detecting humoral (total IgE) and cellular (eosinophilic and basophilic granulocyte count, eosinophil cationic protein) mediators of allergic reaction; 2) identify triggers of allergic reaction (specific IgE to particular causative allergens, tests for basophilic granulocyte *ex vivo* activation, leukotrienes, CD63, histamine); 3) assess the clinical course of allergic reaction (tryptase for immediate reaction, eosinophil cationic protein for delayed reaction); 4) enable specific immunotherapy monitoring (total and specific IgE, specific IgG4 and IgG1, IgG4/IgG1 ratio, proallergic and proinflammatory cytokines); and 5) estimate diagnostic efficiency of determination of particular humoral or cellular mediators of allergic reaction (i.e., sensitivity, specificity, positive and negative predictive value).

Althouh laboratory investigations are a useful tool in the diagnosis and management of allergic diseases many problems remain unsolved. Those problems can be grouped into three categories: preanalytical (specimen collection), analytical and post-analytical (interpretation, follow-up, retesting) phases of laboratory testing.

Keywords: allergy; IgE; basophils; leukotriene; immunotherapy

The requests made to clinical biochemists for laboratory diagnosis of allergic diseases refer to a battery of tests that should provide the following: 1) determine the type of allergic reaction by detecting humoral and cellular mediators of allergic reaction; 2) identify triggers of allergic reaction; 3) assess the clinical course of allergic reaction (immediate, delayed, or prolonged); 4) enable specific immunotherapy monitoring;

Correspondence to: Doc. dr. sc. Slavica Dodig, Ph. D., Srebrnjak Children Hospital, Srebrnjak 100, 10000 Zagreb, E-mail: slavica@dodig.net

and 5) estimate diagnostic efficiency of determination of particular humoral or cellular mediators of allergic reaction. The tests currently used and their diagnostic value in the detection and therapeutic monitoring of allergic diseases are briefly described.

1. IDENTIFICATION OF THE TYPE OF ALLERGIC REACTION

Identification of the type of allergic reaction implies determination of humoral and cellular allergic reaction mediators, including total immunoglobulin E (IgE) concentration, eosinophilic and basophilic granulocyte count [1], eosinophil cationic protein (ECP) concentration [2], identification of membrane markers of basophil activation (CD63), and histamine release from basophilic granulocytes [3].

1. 1. Determination of total IgE concentration

As a simple and automated method, determination of total IgE concentration in serum is included in the screening work-up for atopy [4]. Serum concentration of IgE antibodies varies from very low levels in infancy through several-fold levels from the age of nine years (Table 1). None of other immunoglobulins undergoes such an increase within the normal range during lifetime.

Table 1. Upper limits of IgE concentration in 95% of children according to age

Age (years)	IgE, 95th centile (kIU/L)
<1	20.2
1	30.4
2	38.6
3	57.0
4	57.2
5	65.4
6	73.0
7	82.6
8	76.4
9	98.4
10	102.6
11	104.8
12	101.4
13	97.4
14	94.2
15	105.2
16	100.0

The upper reference limits of total IgE concentration in children are consistent with cut-off values between atopic (n=4520) and nonatopic (n=3355) subjects. The values were determined at Department of Clinical Laboratory Diagnosis, Srebrnjak Children's Hospital in Zagreb (blood sampling was performed during the 2001-2004 period) [5].

In addition to its role in detecting atopic disease, determination of IgE concentration appears to point to the disease severity, in children below 16 years of age in particular [6,7]; however, some authors would not agree with the latter [8,9].

1. 2. Eosinophilic and basophilic granulocyte count

Although numerous cell types (lymphocytes, eosinophilic granulocytes, mastocytes, basophilic granulocytes, endothelial cells, epithelial cells of the respiratory system, neutrophilic granulocytes, antigen-presenting cells) are involved in allergic reaction, eosinophilic granulocyte count in the blood and nasal swab is mostly determined in daily routine. The advent of blood counters offering automated determination of basophilic granulocyte count has enabled the use of this adjunct parameter in the assessment of allergic disease.

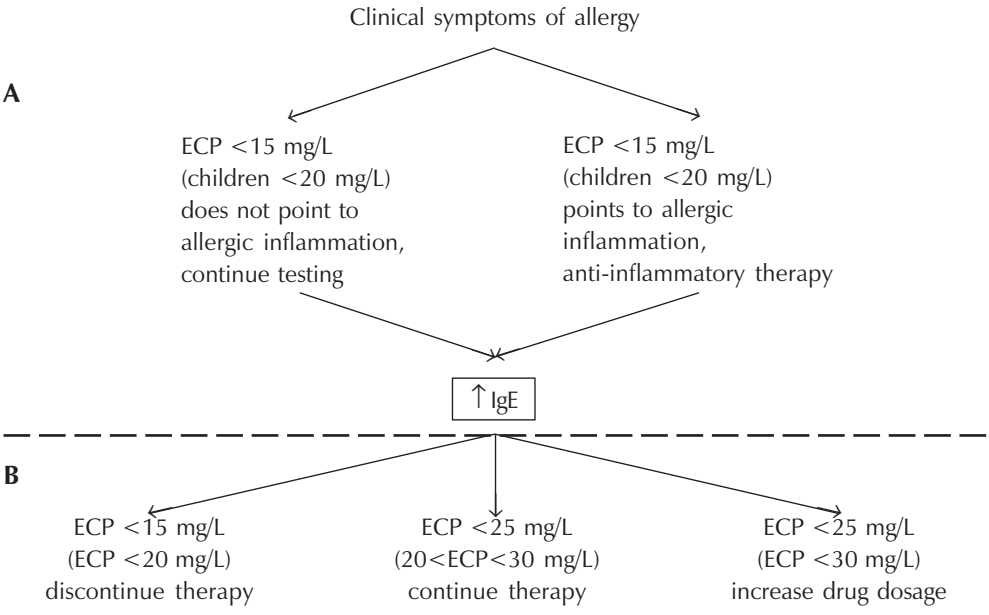

Fig. 1. Assessment of ECP value in monitoring/treatment of allergic inflammation.

1. 3. Eosinophil cationic protein (ECP) concentration

In daily routine, determination of the concentration of ECP released from eosinophilic granulocyte granules allows for the course of allergic reaction to monitor, since an increased ECP concentration (like eosinophilic granulocyte count) points to acute allergen exposure and thus to the severity of allergic inflammation [10,11]. Thus, it can be used as a marker in therapeutic monitoring in asthmatic patients. The upper reference limit is 15 ìg/L for adults and 20 ìg/L for children, and levels exceeding these cut-off values point to allergic inflammation (Fig. 1).

A - Increased serum ECP concentration (>15µg/L in adults and > 20 µg/L in children, respecitvely) provides an additional information to complement the clinical evaluation of allergic inflammation. B - Since therapy with corticosteroids can reduce allergic inflammation, ECP measurement can therefore be used for guiding the anti-inflammatory response. The advice is to check ECP before considering changing the treatment dose. Corticosteroid treatment may be reduced if the patient's clinical situation is stable and the ECP level is normal.

2. DETECTION OF THE TRIGGER OF ALLERGIC REACTION

Blood concentration of specific IgE to particular causative allergens detected by cutaneous testing is determined to identify the trigger of allergic reaction. In early childhood when cutaneous tests are less reliable, determination of IgE to a group of causative allergens (inhalant or nutritional; Phadiatop – Yes or No test for IgE, 93% sensitivity and 89% specificity) is recommended in screening for causative allergens, as an alternative to cutaneous testing [12]. If hypersensitivity is not demonstrated by this procedure, no further testing is required (99% negative predictive value); however, when hypersensitivity to a group of allergens is proven (89% positive predictive value), the procedure should be followed by determination of particular specific IgE in the same serum sample. It should be noted that the values obtained by Phadiatop method are expressed in arbitrary units set by the manufacturer.

2. 1. Determination of allergen-specific IgE concentrations

Upon detection of causative allergens by the skin prick test, the presence of specific IgE against these allergens and their main epitopes is determined in serum of patients with a clinical picture of allergic disease. Determination of specific IgE in serum indicates current immune activity, while skin testing reveals IgE local bioactivity mediated by long-living cells [13]. If the concentration of specific IgE is determined by the standardized method [14] (according to the World Health Organization Second Internation-

Table 2. Serum levels of specific IgE antibodies (CAP procedure) [15]

Specific IgE (kU_A/L)	Class	Result interpretation
<0.35	0	Unmeasurable concentration of specific IgE
0.36-0.7	1	Lowest concentration of specific IgE
0.71-3.5	2	Low concentration of specific IgE
3.6-17.5	3	Moderate concentration of specific IgE
17.6-50.0	4	High concentration of specific IgE
51.0-100.0	5	Very high concentration of specific IgE
>100.0	6	Extremely high concentration of specific IgE

al Reference Preparation for Human IgE, WHO 2nd IRP 75/502), results can be quantitatively expressed within a range from 0.36 to 100 kU_A/L, except for IgE classes 1-6 (Table 2).

Table 3 shows correlation of the results obtained by determination of total and specific IgE in serum and can be used a guide in interpretation of results.

2. 2. Activation of basophilic granulocytes

This group of tests also includes determination of the concentrations of released histamine (radioimmunology, ELISA), leukotrienes (ELISA) [16,17] and surface CD markers (flow cytometry) following basophilic granulocyte activation by the causative aller-

Table 3. Interpretation of *in vitro* procedures

Total IgE (kIU/L)	Specific IgE (kIU_A/L)						
	<0.35	0.36-0.7	0.71-3.5	3.6-17.5	17.6-50.0	51.0-100.0	>100.0
Very low	No atopy	Perhaps not significant; requires repeat testing*	Possible individual hypersensitivity; repeat testing*	Possible individual hypersensitivity	Possible individual hypersensitivity	Possible multiple hypersensitivity	Possible multiple hypersensitivity
Normal	No atopy	Perhaps not significant; requires repeat testing*	Cut-off value; repeat testing*	Possible individual hypersensitivity	Possible individual hypersensitivity	Possible multiple hypersensitivity	Probably multiple hypersensitivity
Increased	Atopy not excluded; expand specific IgE spectrum	Atopy not excluded; expand specific IgE spectrum	Atopy not excluded; expand specific IgE spectrum;	Probably multiple hypersensitivity history	Multiple hypersensitivity	Probably multiple hypersensitivity	Probably multiple hypersensitivity
Very increased	↓	↓	↓	↓	↓	↓	↓

*these findings may be due to the patient's pollen hypersensitivity, therefore repeat testing upon completion of pollination may prove useful

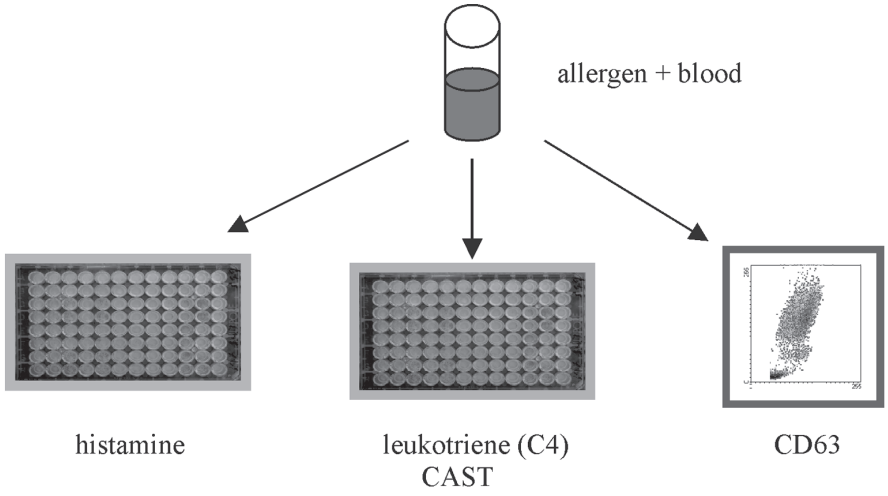

Fig. 2. The principle of methods of basophilic granulocyte activation. Basophilic granulocytes derived from patient blood are activated by the causative allergen *ex vivo,* then various markers of their activation can be determined (CAST = Cellular Allergen Stimulation Test).

gen *ex vivo* (Fig. 2) [18]. Cellular *in vitro* diagnosis has greatly improved with the introduction of the method of flow cytometry. In allergic diseases, the diagnosis based on flow cytometry is for the time being mostly employed in research studies [19]. The role of tests for basophilic granulocyte activation in the diagnosis of allergies remains to be determined.

3. ASSESSMENT OF THE CLINICAL COURSE OF ALLERGIC REACTION

Evaluation of the clinical course of allergic reaction, i.e. immediate, delayed or prolonged hypersensitivity, can theoretically be done by determination of the existing mediators such as tryptase (a marker of anaphylaxis, immediate reaction) or ECP (delayed reaction), or by determination of synthesized mediators of allergic reaction such as leukotrienes following basophilic granulocyte exposure to causative allergens. For the time being, these tests are only performed in research. Tryptase is a serine protease that is released, along with histamine, from mastocytes upon the allergen-IgE complex formation, and represents a marker of anaphylactic reaction [20]. As histamine concentration rises within 15-30 minutes and returns to normal values within one hour, it is quite difficult to measure. Therefore, the measurement of tryptase is used as a more reliable laboratory parameter. Increased tryptase concentration (>13.5 ìg/L) can be demonstrated within 3-6 hours of anaphylactic reaction, then it returns to cut-off values in the next 12-14 hours.

4. SPECIFIC IMMUNOTHERAPY MONITORING

Specific immunotherapy (SIT) *in vitro* monitoring is burdened with a number of problems, from those related to the patient himself and the immune pathomechanisms that have led to the development of allergic disease; coadministration of symptomatic and anti-inflammatory therapy; long-term SIT (currently, no parameters are available to predict with certainty in the early stage of disease whether therapy will eventually prove useful for the patient); choice of biomarkers and defining their cut-off values above or below which there is or there is no protective action of an immune system segment; through laboratory procedures that undergo rapid modifications and advances, thus the results obtained at the beginning of some longitudinal studies cannot be reliably compared with the results recorded at the end of the same studies or with the results reported from other studies; in addition, many of the laboratory procedures that could be used to determine some key biomarkers (e.g., immunoglobulin subclasses, cell subpopulations, cytokines, etc.) cannot be employed in daily routine but remain reserved for scientific research. Besides clinical symptoms, some studies also monitored results of *in vivo* (cutaneous testing) and *in vitro* tests [21,22], e.g., determination of the concentration of total IgE and specific IgE [23], specific IgG [24,25], specific IgG4 and IgG1 (IgG4/IgG1 ratio) [26], IgG2, lymphocyte T subpopulation [27], proallergic cytokines (IL-4, IL-13 and IL-5), proinflammatory cytokines (IFN-γ, IL-2 and IL-12) [28], and leukotrienes [29]. It should not be forgotten that monitoring of the severity of clinical symptoms in each individual patient is of paramount importance in the overall immunotherapy monitoring in patients with allergic diseases.

Lately, recombinant allergens have been used worldwide in the diagnosis and in SIT, enabling determination of patient profile and use of individualized SIT, i.e. only those allergen determinants (epitopes) to which hypersensitivity has developed in the respective patient. Assessment of specific IgE and specific IgG4 concentrations during immunotherapy yields better results with the use of recombinant allergens than with the usual allergen preparations [30].

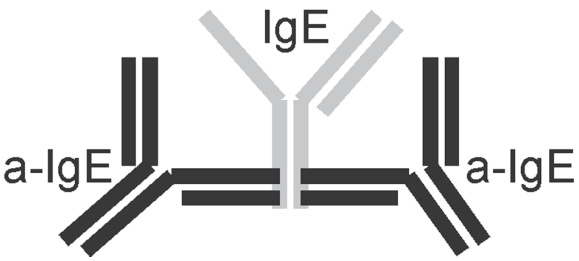

Fig. 3. Anti-IgE (a-IgE) binds free IgE.

The administration of monoclonal anti-IgE (omalizumab) results in free IgE blocking, thus reducing the level of free IgE capable of binding to mastocytes and basophilic granulocytes (Fig. 3) [31]. Therefore, determination of total IgE concentration on the very first day of omalizumab administration is useful to monitor free IgE concentration.

5. ASSESSMENT OF DIAGNOSTIC EFFICIENCY

Diagnostic procedures are expected to be accurate, precise, sensitive, specific, reliable, reproducible, and with minimal interfering factors [32,33]. A prerequisite for the most efficient use of diagnostic tests is to know their limitations, their analytical, preanalytical and postanalytical interferences, and their diagnostic efficiency (i.e. sensitivity, specificity, positive and negative predictive value, table 4). Each healthcare institution should determine diagnostic value of every diagnostic procedure *in vivo* or *in vitro*.

Table 4. Diagnostic efficiency of total and specific IgE (including Phadiatop) and ECP determination (adapted from 34, 35)

Analyte	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Total IgE	97.1	93.3	94.5	96.6
Phadiatop - adults	70.8	90.7	72.6	89.9
Phadiatop - infants	65.0	100.0	100.0	93.0
Specific IgE, asthmatic children (*Dermatophagoides pteronyssinus*)	100.0	96.7	97.3	100.0
ECP, asthmatic children	97.4	100.0	100.0	96.8

6. PROBLEMS IN DIAGNOSIS OF ALLERGY

Althouh laboratory investigations are a useful tool in the diagnosis and management of allergic diseases many problems remain unsolved. Those problems can be grouped into three categories: preanalytical (specimen collection), analytical (testing) and post-analytical (interpretation, follow-up, retesting) phases of laboratory testing.

6. 1. Preanalytical problems

– Time of blood collection for determination of total IgE, specific IgE, ECP and eosinophil count, respectively, could be important in cases of hypersensitivity to:
  • seasonal allergens (level of selected analytes is greater in pollen season),
  • insect sting allergens (waning of allergen-specific IgE with time following exposure), and

- drug allergens (long time elapsed between the administration of the drug and specific IgE determination will result with negative specific IgE).

– The eosiniphil release of ECP in vitro during clotting is time- and temperature-dependent. ECP levels in serum increase with time as the ECP release from the eosinophils will continue until serum is separated from the cells. Therefore the blood collection tube, coagulation time and temperature must be kept within specified limits during clotting.
– Increased concentration of total tryptase activity can be detected up to three to six hours after the anaphylactic reaction. It returns to normal value within 12-14 hours after release. Samples should preferably be collected between 15 minutes and 3 hours after the suspected event causing mast cell activation [20].

6. 2. Analytical problems

Total and specific IgE antibody determination in serum can produce different results depending on the method. Therefore standardized methods should be used.

6. 3. Post-analytical problems

Correlation of *in vitro* testing with *in vivo* testing as well as with clinical finding is the most important for diagnosis and monitoring of allergic disease [8,15]. Some problems can be specialized:

– Under ideal conditions, specific IgE determination should provide results concordant with skin prick testing. However, common allergen extracts are not used in these two methods.
– In spite of the fact that reference intervals for total IgE are defined, 5 % of non-atopic subjects have increased total IgE concentration, and up to 10% of atopic subjecte might have IgE concentration inside the reference intervals [5].
– Sensitisation alone does not indicate clinically significant hypersensitivity. Conversely, a negative result does not necessarily exclude clinically significant allergy.
– No single type of *in vitro* test should be used as the only diagnostic test for food allergy.
– No biomarker can assess clinical course of allergic disease.
– Although effective SIT is accompanied by increase of allergen specific IgG/IgG4 antibodies and decrease of total/specific IgE antibodies [24-26], as yet, no early marker can predict the final outcome of SIT.

In conclusion, it should be noted that clinical biochemistry laboratories in Croatia included in the Croatian Institute of Health Insurance system perform most diagnostic procedures in the field of allergology (e.g., eosinophilic granulocyte count, total and specific IgE concentration, ECP, tryptase) by use of standardized methods, thus making

the values obtained at different laboratories comparable. In addition, these clinical biochemistry laboratories are included in international performance quality assessment, e.g., Quality Club (Pharmacia Diagnostics), thus receiving reports on performance quality in each individual laboratory for particular analytes on a monthly basis.

References

[1] *Prussin C, Metsalfe DD.* IgE, mast cells, basophils, and eosinophils. J Allergy Clin Immunol 2006;117:S450-6.

[2] *Nja F, Røksund OD, Carlsen K-H.* Eosinophil cationic protein (ECP) in schoolchildren living in a mountainous area of Norway: a population-based study of ECP as a tool for diagnosing asthma in children with reference values. Allergy 2001;56:138-43.

[3] *Szegedi A, Irinyi B, Gal M, Hunyadi J, Danko K, Kiss E, Sipka S, Szegedi G, Gyimesi E.* Significant correlation between the CD63 assay and the histamine release assay in chronic urticaria. Br J Dermatol 2006;155:67-75.

[4] *Platts-Mills TAE.* The role of immunoglobulin E in allergy and asthma. Am J Respir Crit Care Med 2001;164:1-5.

[5] *Dodig S, Richter D, Benko B, Živčić J, Raos M, Nogalo B, Čepelak I, Dodig M.* Cut-off values of total serum IgE between nonatopic and atopic children in north-west Croatia. Clin Chem Lab Med 2006;44:639-47.

[6] *Siroux V, Oryszczyn MP, Paty E, Kauffman F, Pison C, Vervloet D, Pin I.* Relationships of allergic sensitization, total immunoglobulin E and blood eosinophils to asthma severity in children of the EGEA Study. Clin Exp Allergy 2003;33:746-51.

[7] *Kovač K, Dodig S, Tješić-Drinković D, Raos M.* Correlation between asthma severity and serum IgE in asthmatic children sensitized to *Dermatophagoides pteronyssinus*. Arch Med Res 2007;38:99-105.

[8] *Duane LS, Stein R, Halonen M, Holberg CJ, Wright A, Martinez FD.* Total serum IgE and its association with asthma symptoms and allergic sensitization among children. J Allergy Clin Immunol 1999;104:28-36.

[9] *Laske N, Niggemann B.* Does the severity of atopic dermatitis correlate with serum IgE levels? Pediatr Allergy Immunol 2004;15:86-8.

[10] *Prehn A, Seger RA, Faber J, Torresani T, Molinari L, Gerber A, Senhauser FH.* The relationship of serum-eosinophil cationic protein and eosinophil count to disease activity in children with bronchial asthma. Pediatr Allergy Immunol 1998;9:197-203.

[11] *Venge P.* Serum measurements of eosinophil cationic protein (ECP) in bronchial asthma.Clin Exp Allergy 1993:23(Suppl 2):3-7.

[12] *Ballardini N, Nilsson C, Nilsson M, Lilja G.* ImmunoCAP Phadiatop Infant – a new blood test for detecting IgE sensitisation in children at 2 years of age. Allergy 2006;61:337-43.

[13] *Ahlstedt S.* Understanding the usefulness of specific IgE tests in allergy. Clin Exp Allergy 2002;32:11-6.

[14] *Johansson SGO.* ImmunoCAP. Specific IgE test: an objective tool for research and routine allergy diagnosis. Expert Rev Mol Diagn 2004;4:273-9.

[15] *Plebani M.* Clinical evaluation and cost-benefit analysis of a new system (Pharmacia CAP system) for the identification of specific IgE. Uppsala: Pharmacia Diagnostics AB, 52-5103-40/01, 1991.

[16] *de Weck AL, Sanz ML.* Cellular Allergen Stimulation Test (CAST) 2003, a review. J Invest Allergol Clin Immunol 2004;14:253-73.

[17] *Rudolf M, Milavec-Puretić V, Lipozenčić J, Malenica B.* Clinical usefulness of cellular antigen stimulation test in detection of aspirin allergy. Acta Dermatovenerol Croat 2005;13:93-6.

[18] *Demoly P, Lebel B, Arnoux B.* Allergy review series X: Progress in diagnosis of allergy *in vitro* – allergen induced mediator release tests. Allergy 2003;58:553-8.

[19] *Dekaris D. In vitro* dijagnostika celularnih imunoreakcija u alergijskim bolestima dišnog sustava. Medicus 1997;6:45-53.

[20] *Edston E, van Hage-Hamsten M, Johansson SG.* Tryptase – at last a useful diagnostic marker for anaphylactic death. Allergy 1996;51:443-4.

[21] *Bousquet J, Calvayrac P, Guerin B* et al. Immunotherapy with standardized *Dermatophagoides pteronyssinus* extract. I. *In vivo* and *in vitro* parameters after a short course of treatment. J Allergy Clin Immunol 1985;76:734-44.

[22] *Crameri R.* Allergy diagnosis, allergen repertoires, and their implications for allergen-specific immunotherapy. Immunol Allergy Clin North Am 2006;26:179-89.

[23] *Raos M, Dodig S, Koncul I, Trešćec A.* Praćenje učinkovitosti imunoterapije astme u djece u *in vivo* i *in vitro* testu. Paediatr Croat 1993;37:71-3.

[24] *Einarsson R, Dreborg S, Hammarstrom L, Lofkvist T, Smith SI, Svensson G.* Monitoring of mite *Dermatophagoides farinae* allergen-specific IgG and IgG subclass distribution in patients on immunotherapy. Allergy 1992;47:76-82.

[25] *Akdis M, Akdis CA.* Mechanisms of allergen-specific immunotherapy. J Allergy Clin Immunol 2007;119:780-91.

[26] *Gehlhar K, Schlaak M, Becker WM, Bufe A.* Monitoring allergen immunotherapy of pollen-allergic specific IgG4 to IgG1 correlates with clinical outcome. Clin Exp Allergy 1999;29:497-506.

[27] B*enjaponpitak S, Oro A, Maguire P, Marinkovich V, De Kruyff RH, Umetsu DT.* The kinetics of change in cytokine production by CD4 T cells during conventional allergen immunotherapy. J Allergy Clin Immunol 1999;103:468-75.

[28] *Nouri-Aria KT, Wachholz PA, Francis JN, Jacobson MR, Walker SM, Wilcock LK, Staple SQ, Aalberse RC, Till SJ, Durham SR.* Grass pollen immunotherapy induces mucosal and peripheral IL-10 responses and blocking IgG activity. J Immunol 2004;172:3252-9.

[29] *Kopp MV, Mayatepek E, Engels E, Brauburger J, Riedinger F, Ihorst G, Wahn U, Kuehr J.* Urinary leukotriene E4 levels in children with allergic rhinitis treated with specific immunotherapy and anti-IgE (Omalizumab). Pediatr Allergy Immunol 2003;14:401-4.

[30] *Mothes N, Valenta R, Spitzauer S.* Allergy testing: the role of recombinant allergens. Clin Chem Lab Med 2006;44:125-32.

[31] *Beeh KM, Beier J, Buhl R.* Saisonale Schwankungen des Serum-IgE-Spiegels und deren mögliche Bedeutung für die Berechnung der therapeutischen Dosis von Omalizumab (rhuMab-E25, anti-IgE). Pneumologie 2004;58:546-51.

[32] *Williams PB, Barnes JH, Szeinbach SL, Sullivan TJ.* Analytic precision and accuracy of commercial immunoassays for specific IgE: establishing a standard. J Allergy Clin Immunol 2000;105(6 Pt 1):1221-30.

[33] *Dolen WK.* IgE antibody in the serum – detection and diagnostic significance. Allergy 2003;58:717-23.

[34] *Vidal C, Gude F, Boquete O, Fernandez-Merino MC, Meijide LM, Rey J, Lojo S, Gonzales-Quintela A.* Evaluation of the Phadiatop test in the diagnosis of allergic sensitization in a general adult population. J Invest Allergol Clin Immunol 2005;15:124-30.

[35] *Nogalo B.* Ispitivanje dijagnostičke osjetljivosti, specifičnosti i djelotvornosti testova *in vivo* i *in vitro* u djece preosjetljive na inhalacijske alergene. MS thesis. Zagreb: Zagreb University School of Medicine, 2005.

Sažetak

Suvremena laboratorijska dijagnostika alergije

Zahtjevi laboratorijske dijagnostike alergijskih bolesti odnose se na odabir analiza koje bi trebale: 1. odrediti vrstu alergijske reakcije otkrivanjem humoralnih (ukupni IgE) i staničnih (broj eozinofilnih odnosno bazofilnih granulocita, eozinofilni kationski protein); 2. otkriti pokretače alergijske reakcije (specifični IgE na pojedinačne uzročne alergene, testovi *ex vivo* aktivacije bazofilnih granulocita – leukotrijeni, CD63, histamin); 3. procijeniti klinički tijek reakcije (triptaza za ranu reakciju, eozinofilni kationski protein za kasnu reakciju); 4. omogućiti praćenje uspješnosti specifične imunoterapije (ukupni i specifični IgE, specifični IgG4 i IgG1, omjer IgG4/IgG1, proalergijski i proupalni citokini); 5. odrediti dijagnostičku djelotvornost određivanja pojedinih humoralnih ili staničnih posrednika alergijske reakcije (osjetljivost, specifičnost, pozitivna i negativna, predvidljiva vrijednost).

Iako su laboratorijska istraživanja korisna u dijagnozi i liječenju alergijskih bolesti, brojni problemi još su neriješeni. Ti se problemi mogu svrstati u tri glavne kategorije: preanalitička (uzorkovanje), analitička i poslijeanalitička (tumačenje, praćenje, ponavljno testiranje) faza laboratorijskog određivanja.

Ključne riječi: alergija; IgE; bazofilni granulociti; leukotrijeni; imunoterapija